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Short-term immunogenicity and safety of hepatitis-A and varicella vaccines in HIV-exposed uninfected and HIV-unexposed South African children

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ABSTRACT

Background: HIV-exposed uninfected (HEU) children have increased risk of infectious morbidity during early childhood. We evaluated the short-term immunogenicity and safety of single dose inactivated hepatitis-A virus (HAV) vaccine and live attenuated varicella zoster virus (VZV) vaccine in South African children.

Methods: 195 HIV-unexposed and 64 HEU children received either one dose of HAV or VZV vaccine at 18 months of age. Blood samples were tested for hepatitis-A or VZV antibodies before and one month after vaccination by chemiluminescent microparticle immunoassay and enzyme-linked immunosorbent assay, respectively. All children were evaluated for solicited adverse events (AEs).

Results: One-month post-vaccination, a similar percentage of HIV-unexposed (91.8%) and HEU (82.9%) children were seropositive for hepatitis-A ($p = 0.144$). VZV antibody geometric mean fold-rise was also similar in HIV-unexposed (5.6; 95%CI: 4.6–6.7) and HEU children (5.1; 95%CI: 3.7–7.2); however, only 44% HIV-unexposed and HEU seroconverted (titers > 50 mIU/ml). AEs occurred with similar frequency and severity between groups, except for more systemic AEs after VZV vaccination in HEU than HIV-unexposed children.

Conclusions: Single dose HAV and VZV vaccine was similarly immunogenic in HIV-unexposed and HEU children. We did not identify differences in short-term humoral immunity after administration of either a live attenuated or inactivated vaccine. Seroconversion rates after a single dose of VZV vaccine were, however, lower compared to reports from previous studies (85–89%).

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1. Introduction

Prevention of mother-to-child HIV transmission programs have reduced vertical transmission of HIV to newborns, however, there remains a growing population of children born to women with HIV but who are not infected by HIV (i.e. HIV-exposed uninfected; HEU) [1]. HEU children have increased risk of infectious morbidity and mortality compared with HIV-unexposed children, particularly in early childhood [2,3].

Hepatitis-A virus (HAV) is a common cause of viral hepatitis, particularly in low- and middle-income countries [4]. The global incidence of acute hepatitis-A has increased from 150 (141–159) million in 1990 to 170 (161–180) million in 2017 [5]. Most children are exposed to HAV before 5 years of age when hepatitis-A infection commonly has an asymptomatic/mild clinical course [6]. African countries may, however, be experiencing an epidemiological transition to lower HAV endemicity [7–10], which

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is associated with absence of HAV infection during early childhood and predisposition to more severe illness when acquired later in life [6]. In South Africa, sero-epidemiological studies indicate intermediate HAV endemicity between 2005 and 2015, with total antibody positivity of 53% at 1–4 years of age and > 90% after 25 years of age [10]. This indicates a need to consider the World Health Organization (WHO) recommendation for inclusion of HAV vaccination in South Africa's national immunization program for children ≥ 1 year of age [6,10]. Given the sizeable number of HEU children, it is important to study HAV vaccination in this group.

Although two doses of Hepatitis A vaccine are traditionally recommended, similar immune responses have been achieved after a single compared to two doses [11,12] and single dose vaccination is programmatically more realistic and feasible. To our knowledge, immunogenicity data following a single dose of inactivated HAV vaccine regimen in HEU children are limited to one Brazilian study [13]. Seroconversion (antibody titer > 20 mIU/ml as measured by enzyme-linked immunosorbent assay) was reported in 72% (18/25) of HEU children following HAV vaccination at mean 5.1 years of age [13].

Varicella infection is usually self-limited, but may cause serious complications, including secondary bacterial infections, pneumonia or encephalitis [14]. Differences in epidemiology between temperate and tropical climates have been described, with primary infection occurring at older age in tropical climates, thereby increasing the risk of developing complications [15]. Safe and effective vaccines against varicella and herpes zoster are available [16]. The WHO recommends immunization with a live attenuated varicella zoster virus (VZV) vaccine at 12–18 months of age in settings where varicella is of public health importance [16]. Although VZV immunization has been introduced in the national vaccination programmes of several countries [15,17,18], it is seldom used in Africa [14] and may be of use in South Africa. Unusual clinical manifestations, such as haemorrhagic varicella, have been reported in HEU children in South Africa [3]. To our knowledge, there are no VZV vaccine studies in HEU children from sub-Saharan Africa.

This study evaluated the short-term immunogenicity and safety of a single dose of HAV and VZV vaccines administered to HIV-unexposed and HEU South African children at 18 months of age.

2. Methods

2.1. Study design and setting

This prospective cohort study enrolled HIV-unexposed and HEU children aged 18 weeks of age at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa between April 2017 and February 2019. All children participating in the present study were also evaluated for measles vaccination antibody response and these results have been published separately [19]. Here we report on the immune response to varicella and hepatitis-A vaccination (NCT03330171). HIV-unexposed children who were additionally enrolled in a randomized, open-label trial evaluating reduced dosing schedules of pneumococcal conjugate vaccine (PCV) (NCT02943902) were invited to participate in consecutive order. All participants were recruited from the same population during the same time period. Eligible participants were those born at ≥ 37 weeks gestation, birth weight > 2499 g, healthy based on medical history and physical examination, and absence of previous varicella or hepatitis-A disease or vaccination since birth. Inclusion and exclusion criteria are detailed in Supplementary data 1.

2.2. Procedures

Participants in the measles study were allocated consecutive study identifiers. Study staff and participants were not blinded. For the current study, half of the children participating in the measles study received either one dose of inactivated HAV vaccine (AVAXIM® - Pediatric, 80 U/0.5 mL, Sanofi Pasteur, Lyon, France) intramuscularly or live attenuated VZV vaccine (VARILRIX®, GlaxoSmithKline, Rixensart, Belgium) subcutaneously in the deltoid region at 18 months of age (547 days ± 14) based on having an even or odd numeric study identifier. The vaccines were stored at 2–8 °C prior to use. Parents were asked about compatible illness prior to vaccine administration and received hepatitis-A vaccine if chicken pox was reported. All children received other childhood vaccines according to the South African public immunization programme, except for HIV-unexposed children being randomized to different dosing schedules of PCV during the first year of life. Children were observed for 30 min after vaccination to monitor for anaphylactic reactions. Venous blood specimens were obtained immediately before (18 months) and one-month after vaccination (19 months, 28–35 days post-vaccination).

2.3. Safety assessment

Vaccine safety was assessed using report cards. Parents were trained to record any solicited local (pain/tenderness at injection site, redness, swelling and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) reactions for 7 days following vaccination. Monitoring for serious adverse events (SAEs) was done during one-month post-vaccination, through passive surveillance.

2.4. Laboratory methods

Blood samples were centrifuged and sera were stored at −70 °C until testing. HAV antibodies were measured using a chemiluminescent microparticle immunoassay (Abbott ARCHITECT® HAVAb- IgG). IgG results were calculated by dividing relative light units of the sample by relative light units of the cut-off (S/CO). Seropositivity was defined as S/CO ≥ 1.00 and seroconversion as a change from nonreactive (S/CO < 1.00) pre-vaccination to reactive (S/CO ≥ 1.00) post-vaccination, as per manufacturer's instructions [20]. Further details are provided in Supplementary data 2.

VZV antibodies were measured by commercial enzyme-linked immunosorbent assay (ELISA) (SERION ELISA *classic* Varicella-Zoster IgG, Institut Virion\Serion GmbH, Würzburg, Germany) according to manufacturer's instructions. Following recommendations by Sauerbrei and colleagues for measuring post-vaccination VZV responses using this ELISA kit, positive results were defined as > 50 mIU/ml [21]. Seroconversion was defined as a change from seronegative (≤50 mIU/ml) pre-vaccination to seropositive (>50 mIU/ml) post-vaccination.

2.5. Statistical analyses

With a sample size of 100 HIV-unexposed and 35 HEU children that was calculated for the measles study, this study had 80% power to detect an 18% lower seropositivity after HAV vaccination in HEU children compared to HIV-unexposed children, assuming post-vaccination seropositivity rate of 95% in HIV-unexposed children. This study was also adequately powered at 80% to detect a reduction in seropositivity of at least 25% after VZV vaccination in HEU children compared to HIV-unexposed children, assuming a seropositivity rate of 85% in HIV-unexposed children (premised on 85–89% of children to have gpELISA titers ≥ 5 units/ml after single dose VZV vaccine [16,22]).

HAV IgG antibody responses were compared between groups after controlling for pre-vaccination antibody levels (post-vaccination analysis only). Geometric mean titers (GMT) following VZV vaccination were calculated following $\log_{10}$ transformation of ELISA titer values and were compared between groups using multiple linear regression adjusting for maternal age at delivery and pre-vaccination antibody levels (post-vaccination analysis only). Geometric mean fold-rise (GMFR) of VZV antibody was calculated as the geometric mean of the ratio of post-vaccination to pre-vaccination titers, and compared using multiple linear regression with maternal HIV status, maternal age and pre-vaccination titers as covariates. VZV IgG antibody increase was also assessed as ≥ 2, ≥ 3 and ≥ 4 fold-rise from baseline. Percentage of children meeting serological cut-offs was presented with exact binomial 95% confidence intervals (CI) and compared between groups using multivariable logistic regression adjusting for the above mentioned covariates.

Safety analyses evaluated the proportion of children with at least one adverse event, severe adverse events and SAEs. Categorical variables were compared between groups using Chi-square test and Fisher's exact test and continuous variables using the Student's *t*-test or Mann-Whitney *U* test. Analyses were performed using Stata13 (StataCorp, LP, Texas, USA).

2.6. Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M170276), South Africa. Written informed consent was obtained from all parents or guardians prior to enrolment.

3. Results

3.1. Demographic characteristics

100 HIV-unexposed and 35 HEU children received one dose of HAV vaccine. Two HIV-unexposed participants missed post-HAV vaccine blood collection (withdrawal n = 1, loss to follow-up n = 1). 95 HIV-unexposed and 29 HEU children received one dose of VZV vaccine. Four participants (3 HIV-unexposed and 1 HEU) were unavailable for follow-up serology after VZV immunization (withdrawals n = 2, missing blood samples n = 2). Table 1 describes the demographic characteristics of the participants, who were 18.0 months of age (interquartile range [IQR] 18.0–18.1) at vaccination and 19.0 months of age (IQR 19.0–19.1) at the post-vaccination serology visit. Characteristics were similar between groups, except for HEU children who received VZV vaccine having older mothers; Table 1. Demographics of HIV-unexposed children who received either hepatitis-A vaccine or VZV vaccine were not significantly different from those who enrolled in the main PCV parent study (Supplementary Tables 2 and 3).

3.2. Hepatitis-A vaccine immunogenicity

Overall 5.2% (95% CI: 2.1–10.4) of children were seropositive for HAV pre-vaccination, including 6.0% (95% CI: 2.4–12.6) and 2.9% (95% CI: 0.1–14.9) in HIV-unexposed and HEU children, respectively; Table 2. Prior to HAV vaccination, the median HAV antibody S/CO was 0.21 (IQR 0.16–0.32) in HIV-unexposed and 0.20 (IQR 0.15–0.30) in HEU children.

One month after administration of HAV vaccine, overall 89.5% (95% CI: 83.0–94.1) of children had seropositive titers, including 91.8% (95% CI: 84.5–96.4) of HIV-unexposed and 82.9% of HEU children (95% CI: 66.4–93.4; p = 0.144); Table 2. Of the 126 children seronegative before vaccination, the majority (88.9%; 95% CI: 82.1–93.8) seroconverted after HAV vaccination (91.3% HIV-unexposed and 82.4% HEU; p = 0.196). Post-vaccination, the median S/CO was 3.17 (IQR 2.41–4.38) in HIV- unexposed and 2.98 (IQR 1.66–4.17) in HEU children.

3.3. Varicella vaccine immunogenicity

Prior to vaccination in analyses adjusted for maternal age, GMTs were similar between HIV-unexposed (8.1 mIU/ml; 95% CI: 7.2–9.2) and HEU (9.0 mIU/ml; 95% CI: 6.2–13.2) children (p = 0.373). Three children (2 HIV-unexposed and 1 HEU) had VZV antibody titers > 50 mIU/ml and were excluded from post-vaccination analyses; Table 3.

One-month after VZV vaccination, GMTs increased to 41.6 mIU/ml (95% CI: 34.4–50.3) in HIV-unexposed and to 38.6 mIU/ml (95% CI: 27.8–53.7) in HEU children; Table 3. GMFR was similar in HIV-unexposed (5.6; 95% CI: 4.6–6.7) and HEU (5.1; 95% CI: 3.7–7.2) children in analyses adjusted for maternal age and pre-vaccination titers. The proportion of seropositive children

Table 1
Demographics and study participants' characteristics.

Characteristic	Hepatitis-A vaccine recipients			Varicella vaccine recipients		
	Total n = 135	HIV-unexposed n = 100	HIV-exposed uninfected n = 35	Total n = 124	HIV-unexposed n = 95	HIV-exposed uninfected n = 29
Male, n (%)	75 (56)	56 (56)	19 (54)	54 (44)	43 (45)	11 (38)
Race	135	100 (1 0 0)	35	122	93	29
Black African, n (%)	(1 0 0)	0	(1 0 0)	(98)2	(98)2	(1 0 0)0
Mixed ancestry, n (%)	0		0	(2)	(2)	(0)
Median birthweight, grams (IQR)	3090 (2810–3420)	3120 (2840–3428)	3070 (2790–3410)	3288 (2985–3463)	3280 (2930–3475)	3305 (3095–3425)
Median weight at vaccination, kilograms (IQR)	10.6 (9.6–11.6)	10.6 (9.6–11.7)	10.6 (10.0–11.5)	10.5 (10.0–11.8)	10.8 (10.0–11.9)	10.3 (10.0–11.4)
Median maternal age at delivery, years (IQR)	28.5 (23.4–32.9)	28.0 (22.3–32.6)	29.3 (25.8–34.9)	27.9 (25.1–33.4)	27.0 (24.6–31.4)[a]	33.4 (27.1–39.0)[a]
Median age at vaccination and serology pre-vaccination, months (IQR)	18.0 (18.0–18.1)	18.0 (17.9–18.1)[b]	18.0 (18.0–18.1)[b]	18.0 (18.0–18.1)	18.0 (18.0–18.1)	18.0 (18.0–18.1)
Median age at serology following vaccination, months (IQR)[c]	19.0 (19.0–19.2)	19.0 (19.0–19.2)	19.0 (19.0–19.2)	19.0 (19.0–19.1)	19.0 (19.0–19.1)	19.0 (19.0–19.2)

Abbreviations: IQR, interquartile range;
[a] p-value = 0.002;
[b] p-value = 0.037;
[c] total of 133 children available at post hepatitis-A vaccination visit: 98 HIV-unexposed and 35 HIV-exposed uninfected; total of 122 children available at post varicella vaccination visit: 93 HIV-unexposed and 29 HIV-exposed uninfected;

Table 2
Hepatitis-A IgG antibody response and proportion of children achieving seropositive titers and seroconversion.

Measure	Total	HIV-unexposed	HIV-exposed uninfected	p-value
Pre-vaccination				
Seropositive[a], n/N	7/135	6/100	1/35	0.974
% (95% CI)	5.2 (2.1–10.4)	6.0 (2.4–12.6)	2.9 (0.1–14.9)	
S/CO, median (IQR)	0.21 (0.16–0.30)	0.21 (0.16–0.32)	0.20 (0.15–0.30)	0.860
Post-vaccination				
Seropositive[a], n/N	119/133	90/98	29/35	0.144
% (95% CI)	89.5 (83.0–94.1)	91.8 (84.5–96.4)	82.9 (66.4–93.4)	
Seroconversion[b], n/N in HAV seronegative	112/126	84/92	28/34	0.196
% (95% CI)	88.9 (82.1–93.8)	91.3 (83.6–96.2)	82.4 (65.5–93.2)	
S/CO, median (IQR)	3.06 (2.30–4.34)	3.17 (2.41–4.38)	2.98 (1.66–4.17)	0.160

Abbreviations: CI, confidence interval; HAV, hepatitis-A virus; IQR, interquartile range; S/CO, sample signal to cut-off ratio;
P-values were calculated by Mann-Whitney or Fisher's exact test (pre-vaccination) and linear or logistic regression adjusting for baseline antibody values (post-vaccination);
[a] Seropositivity was defined as sample signal to cut-off ratio (S/CO) $\geq$ 1.00 per manufacturer's specification;
[b] Seroconversion was defined as a change from S/CO < 1.00 to S/CO $\geq$ 1.00.

Table 3
Varicella zoster virus IgG antibody response and proportion of children achieving seropositive titers and seroconversion.

Measure	Total	HIV-unexposed	HIV-exposed uninfected	p-value
Pre-vaccination	n = 124	n = 95	n = 29	
GMT (95% CI)	8.3 (7.3–9.5)	8.1 (7.2–9.2)	9.0 (6.2–13.2)	0.373
Seronegative ($\leq$50 mIU/ml), n	121	93	28	0.524
% (95% CI)	97.6 (93.1–99.5)	97.9 (92.6–99.7)	96.6 (82.2–99.9)	
Seropositive (>50 mIU/ml)[a], n	3	2	1	0.524
% (95% CI)	2.4 (0.5–6.9)	2.1 (0.2–7.4)	3.4 (0.1–17.8)	
Post-vaccination[a]	n = 117	n = 90	n = 27	
GMT (95% CI)	40.9 (34.8–48.1)	41.6 (34.4–50.3)	38.6 (27.8–53.7)	0.743
Seronegative ($\leq$50 mIU/ml), n	65	50	15	0.536
% (95% CI)	55.5 (46.1–64.7)	55.6 (44.7–66.0)	55.6 (35.3–74.5)	
Seropositive (>50 mIU/ml), n	52	40	12	0.536
% (95% CI)	44.4 (35.3–53.9)	44.4 (34.0–55.3)	44.4 (25.5–64.7)	
Change from pre- to post-vaccination				
GMFR[b] (95% CI)	5.5 (4.6–6.4)	5.6 (4.6–6.7)	5.1 (3.7–7.2)	0.743
Seroconversion[c], n	52	40	12	0.536
% (95% CI)	44.4 (35.3–53.9)	44.4 (34.0–55.3)	44.4 (25.5–64.7)	
$\geq$2 fold-rise from baseline, n	103	79	24	0.817
% (95% CI)	88.0 (80.7–93.3)	87.8 (79.2–93.7)	88.9 (70.8–97.6)	
$\geq$3 fold-rise from baseline, n	94	73	21	0.590
% (95% CI)	80.3 (72.0–87.1)	81.1 (71.5–88.6)	77.8 (57.7–91.4)	
$\geq$4 fold-rise from baseline, n	80	63	17	0.824
% (95% CI)	68.4 (59.1–76.7)	70.0 (59.4–79.2)	63.0 (42.4–80.6)	

Abbreviations: CI, confidence interval; GMFR, geometric mean fold-rise; GMT, geometric mean titer;
P-values were either calculated by linear or logistic regression and adjusted for maternal age at delivery and baseline antibody levels (post-vaccination comparison only);
[a] Three participants (2 HIV-unexposed and 1 HEU) with seropositive titers pre-varicella vaccination are excluded from post-vaccination analyses;
[b] Geometric mean of the ratio of post-vaccination titer to the pre-vaccination titer;
[c] Change from $\leq$ 50 mIU/ml pre-vaccination to > 50 mIU/ml post-vaccination.

increased to 44.4% (95% CI: 35.3–53.9) in both groups and less than half seroconverted (44.4% HIV-exposed and 44.4% HEU). Most children (87.8% HIV-unexposed and 88.9% HEU) had at least a 2-fold rise in VZV antibody titer post-vaccination; Table 3.

3.4. Hepatitis-A vaccine safety

Solicited local and systemic reactions occurred with similar frequency and severity in HIV-unexposed and HEU children; Table 4 and Supplementary table 4. There were no vaccine-related adverse events. One SAE (hospitalization for herpetic gingivostomatitis) occurring 24 days following HAV vaccination in an HIV-unexposed child was reported, which completely resolved.

3.5. Varicella vaccine safety

During the seven days following vaccination, 27% HIV-unexposed and 18% HEU experienced $\geq$ 1 local adverse reaction, whereas 57% HIV-unexposed and 29% HEU (p-value = 0.007) experienced $\geq$ 1 systemic adverse reaction; Table 4. Overall, the frequency and severity of solicited adverse events were similar between groups, except for decreased appetite, which was 22% in HIV-unexposed and 14% in HEU (p-value = 0.026); Supplementary table 4. No vaccine-related adverse events and no SAEs were reported during the 28 days following vaccination.

4. Discussion

This study demonstrated that a single dose of inactivated HAV vaccine or live attenuated VZV vaccine administered at 18 months of age was similarly immunogenic in both HIV-unexposed and HEU South African children, albeit seroconversion rates after VZV vaccination were lower than expected. Our study fills a data gap in providing evidence on HAV and VZV immunization in HEU children.

We found no statistical difference in the percentage of HEU children to be HAV seropositive post-vaccination compared with HIV-unexposed children. Our findings of 82.9% seropositivity and 82.4% seroconversion in HEU were slightly higher than the previous study on single dose HAV vaccine in HEU children, in which

Table 4
Reported adverse events following single dose hepatitis-A or varicella vaccination by HIV-exposure.

	Hepatitis-A vaccine recipients		Varicella vaccine recipients	
n/N (%)	HIV-unexposed	HIV-exposed uninfected	HIV-unexposed	HIV-exposed uninfected
Solicited reactions 0–7 days after vaccination				
Local reactions				
≥1	25/100 (25)	10/35 (29)	25/94 (27)	5/28 (18)
Severe	0/100 (0)	2/35 (6)	2/94 (2)	1/28 (4)
Systemic reactions				
≥1	47/100 (47)	15/35 (43)	54/94 (57)[a]	8/28 (29)[a]
Severe	3/100 (3)	4/35 (11)	6/94 (6)	4/28 (14)
Unsolicited serious adverse event after vaccination				
Serious AE ≤ 28 days after injection	1/100 (1)[b]	0/35 (0)	0/94 (0)	0/28 (0)
Vaccine-related serious AE	0/100 (0)	0/35 (0)	0/94 (0)	0/28 (0)

Abbreviations: AE, adverse events; N, total number of participants with vaccination report card / serious adverse event assessment; n, number of participants having a reaction;
[a] p = 0.007;
[b] Participant hospitalized with herpetic gingivostomatitis.

72.0% HEU children seroconverted 4–8 weeks after vaccination [13].

In the present study, a single dose of HAV vaccine generated seropositive antibody responses in 91.8% (95% CI: 84.5–96.4) of HIV-unexposed children. In comparison, 98.6% of Argentinian children had seropositive titers (≥10 mIU/mL as measured by microparticle enzyme immunoassay) one year after a single dose of the same HAV vaccine administered at 11–23 months of age [12]. Similarly, 95.3% of Chinese children vaccinated at 18–60 months of age had seropositive responses by microparticle enzyme immunoassay one year following inactivated HAV vaccination [23].

Despite 10.5% of children in our study not showing a humoral immune response to HAV vaccination, previous studies showed that children with low or undetectable antibody levels after one HAV dose, measured prior to booster administration, were able to elicit a strong humoral response after booster challenge, indicative of a robust anamnestic response [24]. This memory recall response may reflect residual B-cell response capacity. It has also been shown that a single HAV vaccine dose induces HAV-specific T-cell immunity that persists independently of circulating antibody levels and produces a HAV-specific memory response similar to that induced by natural infection [25]. The T-cell immunity may contribute to protection against hepatitis-A viral infection in children without seroconversion.

HIV-unexposed and HEU children had comparable antibody responses to VZV vaccine. A cross-sectional study from the United States reported 98% (55/56) of HEU children with seropositive titers following one dose of VZV vaccine at median 1.5 (IQR 1.1–3.7) years of age as measured either by whole-infected cell ELISA or gpLISA (cutoffs not mentioned), although relation to vaccination could not definitely be ascertained [26].

Seropositivity rates in our study following a single dose VZV vaccination were strikingly lower than previously reported after a single dose of VZV vaccine in different settings; 85–89% had antibody levels ≥ 5 units/mL (based on gpELISA) and an estimated vaccine efficacy of 94.4% during the 10-years following vaccination [22,27–29]. Our findings were also lower than a South African trial on the immunogenicity of a single dose of VZV vaccine, co-administered with measles/mumps/rubella (MMR) vaccine at 15–18 months in healthy children, which showed a varicella response of 73% to 75% (measured by FAMA ≥ 4 [1/dil]) [30]. We followed Sauerbrei and colleagues' recommendation to use an optimized cutoff value of 50 mIU/ml for assessment of post-vaccine immunity [21]. Despite using this cutoff, seropositivity and seroconversion rates remained<50%, which is lower than reported after one dose of the same vaccine [31–33] or when combined with MMR [34,35]. A literature search did not identify any published study evaluating VZV antibodies following immunization using the same Virion\Serion ELISA kit. An Indian study, using a different commercial ELISA kit, found a similar percentage of children experiencing a 3- or 4-fold increase in VZV-specific IgG titer from baseline (73% and 62%, respectively) in children aged 12 months to 12 years after one dose of the same VZV vaccine [36]. Commercial ELISA may not be sensitive enough to detect seroconversion after vaccination, since it is calibrated for diagnosis of natural infection [37] and may therefore underestimate the immunogenicity of vaccines. The choice for commercial ELISA was investigator-driven and due to limited availability of gpELISA.

Pre-vaccination VZV seroprevalence was low (2%), which is corroborated by a recent systematic review showing seropositivity to VZV antibodies in African children aged 1–12 years of 23% (95% CI 17–30%) [38]. The authors of the review suggested that primary VZV infection occurs at a later age in Africa compared to other regions and noted a positive association between age and VZV seropositivity [38].

Both HAV and VZV vaccines were found to be safe and well tolerated. Following VZV vaccination, more HIV-unexposed children experienced one or more systemic reaction than HEU children. The underlying reason for this difference remains unclear. The percentage of children with any local or systemic solicited reaction was similar to that reported in a South African study co-administering VZV vaccine with MMR (10–68%) [30]. The percentage of children with severe systemic reactions was, however, higher in our study (2–14%) compared to the co-administration study (0–4%), particularly in HEU children. No serious adverse events occurred during the 28 days following vaccination.

Limitations of this study include that our sample size only provided 80% power to detect at least a 20% difference following HAV vaccination between HEU and HIV-unexposed (based on our result of 92% seropositivity in HIV-unexposed). Consequently, we may have missed detecting differences of lesser magnitude in HAV antibody responses between the two groups. Also, due to lower than anticipated response rate to VZV vaccination, the study lacked power for this comparison. With the present sample size and 44% seropositivity in HIV-unexposed children, we were only adequately powered (at 80% power) to detect a difference of at least 27% between HEU and HIV-unexposed children after varicella immunization. Nevertheless, our study still yields important information that needs to be pursued. In addition, our study is limited by the absence of an HIV-positive cohort and short duration of follow-up. Long-term follow-up is currently ongoing. Solicited adverse events were followed-up until day 7 post-vaccination, thereby excluding adverse events with a later onset if not reported

by the parent during the next study visit. Assessment of humoral immunity following VZV immunization was done using a commercially available ELISA kit, which may be less sensitive in post-vaccination samples and could therefore underestimate the antibody response.

Based on the WHO recommendation to integrate universal vaccination against HAV in the national immunization schedules in countries with declining endemicity from high to intermediate, several countries have introduced the HAV vaccine during childhood, which led to a considerable decrease in HAV incidence in both vaccinated and non-vaccinatedgroups [39]. Before universal HAV vaccination can be considered, seroprevalence across different age groups and regions needs to be established, in combination with country-specific cost-effectiveness assessment.

In many low- and middle-income countries (LMIC), other vaccine-preventable diseases with greater public health burden or severity, are prioritized over VZV vaccine. Despite the low morbidity and mortality of VZV, the burden of varicella and herpes zoster on healthcare systems and society, in absence of preventive measures, can be considerable [15]. A retrospective review of admissions to a paediatric isolation facility in Durban, South Africa, demonstrated that varicella accounted for 23% of admissions between 1986 and 1996, with 15% of varicella admission (n = 86) and 75% of varicella deaths (n = 6) being associated with HIV-infection between 1994 and 1996 [40].

A theoretical modelling study claimed that if LMIC introduced a one dose VZV vaccine in children 12–18 months of age with coverage between 20% and 80%, there would be an increased risk of an epidemiological shift to older age at infection and increased mortality [41]. In contrast, epidemiologic data from high-income countries have shown a decrease in varicella incidence in all age groups [42] or under the age of 40 [43] after VZV vaccine introduction in the second year of life and, most importantly, of complications, hospitalizations and overall healthcare costs associated with varicella infections [44]. Before routine VZV vaccination can be implemented, countries first need to consider the burden of varicella disease and predict achievable vaccination coverage [41].

In conclusion, we have shown that a single dose of inactivated HAV vaccine at 18 months of age was safe and resulted in most children becoming HAV seropositive in the short-term. Durability of a single dose HAV vaccine needs to be established. Single dose of live attenuated VZV vaccination was safe, but resulted in seropositivity in less than half of children as measured by a commercially available ELISA. A second dose of VZV is expected to improve rate of seroconversion [22]. Future studies should evaluate long-term humoral and cellular response to HAV and VZV vaccines in the African context, in both HIV-unexposed and HEU children.

Author contributions

EAMLM, MCN and SAM contributed to the conception and design of the study; EAMLM, MCN and SAM oversaw the clinical trial, clinical data collection and clinical data management; SB, BTI, LJ and AK were responsible for clinical aspects of the study; AM was responsible for laboratory management; EAMLM conducted laboratory analyses and wrote the first draft of the paper. All authors contributed to subsequent drafts, read and approved the final version of the report.

CRediT authorship contribution statement

Eleonora A.M.L. Mutsaerts: Conceptualization, Formal analysis, Investigation, Writing - original draft, Project administration. **Marta C. Nunes:** Conceptualization, Writing - review & editing, Supervision. **Sutika Bhikha:** Investigation. **Benit T. Ikulinda:** Investigation. **Lisa Jose:** Investigation. **Anthonet Koen:** Investigation. **Andrew Moultrie:** Resources, Project administration. **Diederick E. Grobbee:** Writing - review & editing. **Kerstin Klipstein-Grobusch:** Writing - review & editing, Supervision. **Adriana Weinberg:** Resources, Writing - review & editing, Supervision. **Shabir A. Madhi:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Weinberg receives research grants from Merck and GSK. Moneys go to the University of Colorado. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.03.045.

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